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FIELD TRIALS OF MOSQUITO REPELLENTS IN FLORIDA, ALASKA, AND CALIFORNIA

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ABSTRACT

Four repellents were tested under different climatic conditions found at Turtle Mound, Florida; Fort Wainwright, Alaska; and Colusa, California. Test repellents cyclo-hexamethylene-carbamide and n-butane-hexamethyleneimine-sulfonamide were found to protect as long as or longer than N,N-diethyl-m-toluamide, the standard military repellent, under all test conditions at equal concentrations. Trioxa-pentadecan-1-01 was statistically no different from deet. Mild temperatures in Alaska compared to Florida and California caused all repellents tested to be effective for 7-9 hours despite heavy mosquito populations.

INTRODUCTION. In areas where malaria, yellow fever, dengue, encephalitis, and other mosquito-borne diseases are endemic, mosquito repellents are important to the health of a mobile military force in situations where usual vector control methods are impractical. In view of increasing ecological restrictions on the use of pesticides and growth regulators in addition to mosquitoes developing resistance to these measures, an effective personal repellent can be an important part of individual protection in an overall program of mosquito control.

As a part of a program to find a longer-lasting mosquito repellent, field trials were conducted in North Carolina so repellent efficacy in laboratory testing could be compared to efficacy under field conditions (Shimmin et al 1974). It was felt that evaluation of candidate repellents under different climatic conditions and against different mosquito populations would be desirable for further comparison to laboratory results. Three different areas were selected: the hot, humid tropical-like summer climate in Florida where Gilbert et al (1957) have reported field testing, the arctic summer climate of Alaska where Gorham (1974) has reported heavy mosquito populations during repellent tests, and the hot, dry summer climate of California where heavy localized mosquito populations were found around irrigated fields. The current paper presents the results of those studies.

MATERIALS AND METHODS. Repellents: Repellent compounds tested were cyclohexamethylene-carbamide (carbamide), 99% pure (Dremova and Smirnova, 1970); n-butane-hexamethyleneimine-sulfonamide, 99% pure (Mushi and Razbegaeva 1966); 3,6,9-trioxapentadecan-1-01 (SRI-6), 96% pure (Skinner et al. 1974); N,N-diethyl-m-toluamide (deet), 95% pure (Eastman Chemicals, Practical Grade).

Test Design: A four-site field test method was employed in all three tests (Shimmin et al. 1974). Adhesive-backed foam strips about 2 cm wide were wrapped around the wrist, upper forearm, and mid-forearm of test participants. The foam strips served as boundaries and protective devices against abrasion for the sites. Foam strips outlined 2 sites on each forearm of every volunteer (4 sites per individual). One of the four test repellents was applied in ethanol solution to each site at a constant concentration per unit of skin area. The area of each site was determined by measuring the forearm circumference at points 3, 12, and 21 cm from the wrist. The surface area of each test site was calculated as the area of a frustrum of a cone: Area = $s \times (R_1 + R_2) \times \pi$ where $s = 8$ cm and R_1, R_2 are the radii at the upper and lower boundaries of the site.

The total area marked off by foam strips was covered with repellent solution for that site. Repellents were applied so no repellent appeared on a given elbow or wrist (right or left) site more than once in any group of four individuals. Moreover, all repellents were paired at least twice on the same forearm among 2 groups of 4. One site on each subject was a deet control. All results were analyzed using

Table 1. Repellent protection times against mosquitoes, Turtle Mound, Florida[1].

Weather	Protection Time in Hours, Mean ± s_d			
	Deet	Carbamide[2]	SRI-6	Sulfonamide[3]
33°C, 80-85% RH, 0-5 mph wind	7.6 ± 1.0	9.8 +	4.5 ± 1.7	7.0 ± 2.5

[1]Repellent applied at 0.48 mg/cm^2; mosquito activity ranged from 1200 to 2000 body landings/hour.

[2]No carbamide-treated sites failed at termination of trial.

[3]One site failed in first exposure.

Table 2. Repellent protection times against mosquitoes, Alaska. [1,2]

June	Weather	Protection Time in Hours, Mean ± s_d			
		Deet	Carbamide	SRI-6	
25	16-26°C, Clear, 85-92% RH	7.4 ± 1.4	7.6 ± 1.0	7.2 ± 1.2	
26	22-25°C, Clear, 39% RH	7.9 ± 0.7	7.8 ± 0.7	7.7 ± 1.2	
27	14-16°C, Rainy, 88% RH	8.6 ± 1.6	9.2 ± 1.5	7.2 ± 1.3	
Mean (24 total)		8.0 ± 1.3	8.2 ± 1.3	7.4 ±	1.2[3]
Ratio to deet			1.03	0.93	

[1]Repellents applied at 0.40 mg/cm^2.

[2]Concentration of sulfonamide was lower than 0.40 mg/cm^2, hence, results are omitted.

[3]Significant at 5% level ($p < 0.05$).

a paired comparison with deet. Statistical significance was determined at the 95% level ($\alpha < 0.05$) by the student's t-distribution and Tukey's w-procedure (Ostle 1963).

After repellent applications, volunteers exercised or engaged in moderate physical activity as described below for each separate location. Head nets and gloves were worn whenever individuals entered mosquito-infested test areas. When a bite was received on any treated site, the individual recorded the site and the time in a notebook. A confirmed bite, one bite followed by a second bite within one-half hour, was the criterion for repellent failure.

Mosquito populations were determined by counting the number of mosquitoes landing on a single individual's body in a specified time interval. This number was then extrapolated to body-landings per hour. Alternately, the number of mosquitoes landing on an untreated control forearm were recorded for an individual who was not participating in the repellent testing. This number was recorded as forearm landings per hour. Mosquitoes were collected for identification from the same individual who was employed in population counts.

Turtle Mound State Park, Florida, was characterized by coastal brackish water and coniferous wooded areas with marsh vegetation. Repellents were applied at a dose of 0.48 mg/cm^2 to 4 subjects at 0700 hrs. Initial exposure to mosquitoes was from 1000 to 1230 hrs in the test area. Participants moved about in the test area to stir up mosquitoes. At 1330 hrs the group ran for 3-minutes to induce sweating and reentered the mosquito-infested area for 1 hour and 15 minutes. Since some repellents were still effective, volunteers hiked on the beach for 1 hour under the hot sun. At 1545 hrs, exposure to mosquitoes began again and continued until termination of testing at 1645 hrs, when one repellent-treated site on each individual was still effectively protected from mosquitoes. Mosquito activity ranged from 1200 to 2000 body-landings per hour.

Ft. Wainwright, Alaska test areas were typically taiga regions in low lying wooded areas between rivers and tundra. Each day repellents were applied at a dose of 0.40 mg/cm^2 to 8 subjects in 2 groups of 4. Sulfonamide had precipitated from solution, and the applied dose was somewhat lower. Exposure to mosquitoes began between 1200 and 1300 hrs after application at 0900 hrs. Continuous heavy populations of mosquitoes were experienced as volunteers hiked, forded streams and fished to simulate moderate field activity. Test areas were located north and east of Ft. Wainwright, Alaska, as follows. 25 June, N 64° 54'W 147° 15' on the Little Chena River; 26 June, N 65° 10' W 145° 10' on the East Fork Chena River; and 27 June, N 64° 18' W 146° 25' on the Chena River. If a repellent still afforded

protection from mosquitoes at the termination of testing between 1700 and 1900 each evening, the time of termination was recorded as the repellent protection time. During June in Alaska, sunset occurred approximately 2300 hrs and sunrise about 0130 hrs, although there was enough light to read a book during the short night.

Colusa, California test areas were located along the banks of irrigation or drainage ditches where mosquito activity was apparent. Repellents were applied at a dose of 0.40 mg/cm^2 to 8 subjects in 2 groups of 4 at 0900 hours. Three replicates were run on 25, 26, and 27 September. Mosquito exposure was accomplished periodically during the day when participants entered mosquito infested areas; however, peak biting activity was observed from 1 hour before to one hour after sunset when repellent failures occurred. Participants recorded numbers of bites received on each site as well as the time. During the day volunteers engaged in hiking. Locations of mosquito exposure included a ricefield, the bank of a creek and a ditch next to the highway near the Colusa airport.

RESULTS. Protection times against mosquitoes afforded by the candidate repellents in Florida, Alaska, and California are indicated in Tables 1, 2 and 4, respectively. Analysis of the results from Turtle Mound (Table 1) indicated that the site treated with carbamide protected for the duration of the trial on all four individuals. Hence, the protection time is recorded as 9.8 plus.

In Alaska constant mosquito pressure was experienced throughout the afternoon. Testing was terminated between 1700 and 1900 hrs each day although the sun had not set. Little or no sweating was observed among the participants because of mild temperatures; consequently, all repellents persisted for a significant period (Table 2). Intermittent light rain on the third day apparently had little effect on repellent protection. Mosquito populations are described in Table 3.

At Colusa no repellent-treated sites received two bites during the daylight testing; hence, the length of repellent protection was from application in the morning until the two-hour period of exposure around sunset. Consequently, all protection periods were restricted to the range 10 to 12 hours. A better means of comparing repellents under these conditions is the use of relative protection from mosquitoes compared to deet (Shuman et al. 1974). The number of bites received on deet-treated sites was considered as the baseline for protection and candidate repellents were evaluated in terms of percentage more protection (or percentage fewer bites) than were received on deet-treated sites (Table 4). Mosquito activity and populations are described in Table 5.

DISCUSSION. In Florida under hot, humid tropical-like conditions, test participants exercised sufficiently to induce profuse sweating. Under these test conditions against a heavy population of *Aedes taeniorhynchus*, carbamide was superior to deet (Table 1), while SRI-6 was somewhat less effective and sulfonamide was approximately equal to deet. This finding is in agreement with two laboratory observations. Higher temperatures favor low volatility repellents like carbamide, sulfonamide, and SRI-6 by raising their evaporation rate and increasing the amount of repellent vapor (Gabel et al. 1976). In addition to the temperature stress, heavy sweating also introduces a water washing

Table 3. Mosquito populations for Alaska field trials.

Species	Collected From Whole Body		
	June 25	June 26	June 27
Aedes communis	30	30	19
Aedes nigripes	-	3	-
Aedes cinereus	-	1	1
Aedes excrucians	-	-	3
Aedes pionips	-	-	1
Ophyra leucostoma[1]	-	1	-
Leptoconops sp.[1]	-	-	1
Mosquito Activity (Body landings/hr)	980	1200	2400

[1]Collected with mosquitoes.

Table 4. Percent repellent protection, Colusa, Calif.[1].

Sept.	Weather	Percent Protection from Biting Compared to Deet			
		Deet[2]	Carbamide	SRI-6	Sulfonamide
25	22°C, 52% RH 5-9 mph wind	0	100	38	77
26	24°C, 50% RH 3-5 mph wind	0	38	62	24
27	29°C, 17% RH no wind	0	76[3]	76[3]	57

[1]Repellents applied at 0.40 mg/cm^2 to 8 subjects, testing took place 10 hours after application in the period one hour before to one hour after sunset; bites on each site were recorded.

[2]Mean number of bites received on the deet-treated site for each individual was 1.3, 2.1, and 2.1 bites/site for 25, 26, and 27 September, respectively.

[3]Statistically different from deet ($p < 0.05$).

Table 5. Mosquito populations for California field trials.[1]

September	Collected from Forearms			Collected from Whole Body		
	25	26	27	25	26	2
Aedes dorsalis	25	38	4	14	20	2
Aedes vexans	1	6	-	1	4	7
Anopheles freeborni	2	1	1	-	1	2
Culex tarsalis	1	1	-	1	2	4
Mosquito activity[2]	116	144	12	68	108	72

[1]Mosquitoes collected in 15 min. from sites indicated.

[2]Landings/hr, a different control was used on forearm collections of 27 Sept; all whole body collections were from the same individual.

stress. Resistance to water is greater for carbamide than for deet (T. Spencer, unpublished results), whereas SRI-6 has somewhat less wash resistance than deet (A. Khan, unpublished results). Hence, one might expect carbamide to persist longer and SRI-6 shorter than deet under a sweating stress (Table 1).

In Alaska the mosquito populations were boreal species, principally *Aedes communis* (Table 3). Despite heavy mosquito activity, deet, carbamide and SRI-6 all afforded protection from mosquitoes for more than 7 hours. Sulfonamide results in this test are not comparable because the repellent had precipitated from the application solution and the applied dose was much less than 0.40 mg/cm^2. When testing was terminated each afternoon, some sites treated with carbamide, deet, and SRI-6 were still protected; therefore, the protection times are underestimated in the Alaska trials (Table 2). Although repellents were applied at 15% lower concentrations in Alaska than in Florida, both deet and SRI-6 exhibited an increased protection period in Alaska compared to Florida. This is attributed to cooler temperatures and the absence of a sweating stress on the treated sites in Alaska; hence, climatic conditions might influence repellent efficacy in the field.

In California the mosquito population was principally *A. dorsalis* and *A. vexans* (Table 5). One species, *Culex tarsalis*, is a known vector of encephalitis, while another species, *Anopheles freeborni*, is a potential malaria-vector. Although mosquito activity was somewhat lower than in Florida or Alaska (Table 4), the presence of these potential vectors was significant. Relative repellent efficacy in these tests was reported in terms of percent protection from mosquito bites compared to deet for the period one hour before until one hour after sunset. Warm, dry weather over the three days of testing (Table 4) introduced a high temperature factor more favorable to the low volatility repellents; moreover, test participants did not sweat significantly in the dry weather in contrast to the sweating stress observed in Florida. Consequently, SRI-6 as well as carbamide and sulfonamide afforded more protection than deet.

In these field trials where abrasion is not a major factor, carbamide provided as much or slightly more protection time from mosquitoes than deet under all three climatic conditions. SRI-6 afforded comparable protection to deet under conditions which did not include heavy sweating stress. Finally, sulfonamide was as effective as deet in Florida and California.

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